JOURNAL OF AGRICULTURAL RESEARCH

VOL. VIII WASHINGTON, D. C., MARCH 12, 1917 NO. 11

SPORE-FORMING BACTERIA OF THE APIARY

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PART I.—DESCRIPTION AND COMPARISON OF SPECIES

INTRODUCTION

In a paper by Lambotte in 1902 (7)[1] the statement is made that "foulbrood" of bees can be produced by feeding the insects cultures of *Bacillus mesentericus vulgatus* grown upon a special medium prepared from the juices of bee larvæ. In explanation it is stated further that, by the use of this special medium, *B. alvei* arises as a special variety of *B. mesentericus vulgatus* and produces, on feeding, the tissue changes characteristic of the disease. Lambotte does not describe the disease adequately to make it possible to determine whether he worked with American foulbrood or European foulbrood, and at the time his paper appeared these two diseases were not differentiated clearly. This comparison of the two organisms disagreed so materially from the observations of the author that feeding experiments with *B. mesentericus vulgatus* were begun in 1911. At the outset it was observed that of the organisms of the *B. vulgatus* group, which are isolated in the course of the examination of specimens of bee comb and brood sent to the Bureau of Entomology for diagnosis, it was not possible to identify all as one species. Consequently, it was realized that the first task would be the identification of the various organisms of this group and a comparison of them, for purposes of easy identification, with the other known spore-forming bacteria of the apiary: *B. alvei*, *B. larvae*, and *B. orpheus*. Only *B. vulgatus*, *B. mesentericus*, and *B. orpheus* will be described in detail. For descriptions of *B. alvei* and *B. larvae* the reader is referred to White (16). The illustrations and summary at the end of the paper should aid in differentiating the five known spore-forming bacteria of the apiary. The results of the feeding experiments are given in Part II of this paper.

[1] Reference is made by number to "Literature cited," p. 419-420.

Journal of Agricultural Research, Washington, D. C.
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Vol. VIII, No. 11
Mar. 12, 1917
Key No. K—51

CULTURES STUDIED

SOURCE.—The cultures studied are obtained in the course of the daily examination of specimens of suspected brood and comb. Cultures can nearly always be obtained by adding bits of comb from any source to the melted agar of petri dishes at pouring. The organisms occur also variously distributed about the hive and can be secured from scrapings of hive covers, bottoms, and walls. They are also found occasionally in considerable numbers in some larvæ, as manifested by the goodly number of colonies occurring on agar plates after the contents of a broken-up larva have been added. Over 30 strains isolated from comb and brood from the various sections of the United States have been cultured and studied. For comparison, one culture of *B. vulgatus* was obtained from the Bureau of Animal Industry and one culture was isolated from potato.

AGE OF CULTURES STUDIED.—The cultures were studied at all ages, from the earliest date at which it is possible to isolate pure cultures and inoculate them into the various culture media up to over 4½ years. The rejuvenation of cultures after the method of Fuller and Johnson (4) was found valuable in obtaining constancy of cultural characteristics.

VARIATION.—From the foregoing statements concerning the age and number of cultures studied, it can be seen that abundant opportunity was afforded for observation, first, of the variation in the same strain due to environmental factors acting over a considerable period of time, and, second, of the variation as exhibited in a number of different strains of the same species. Variation due to both of these factors proved to be considerable, but there is no reason to believe that it is of any greater degree for these organisms than for many other bacterial species (1, 5, 6, 9, 11, 15).

Of the organisms of the *B. vulgatus* group isolated and studied, it seems best for the present to place all under two species only. Most of the organisms isolated are to be classed as *B. vulgatus;* a much smaller number belong to the species *B. mesentericus*. It is realized that further studies may show such great permanent differences in biology as to warrant a separation into still other species, especially of some of the organisms described as *B. vulgatus*. There is, however, one variety from sample of brood 2329[1] which presents such differences in cultural features that it is regarded as at least a special variety of *B. vulgatus*, if not a distinct species. This variety is described later in this paper.

MEDIA USED

REACTION.—All media are made 1.5 per cent acid to phenolphthalein, unless otherwise stated (3).

[1] In the original notes all cultures, as the above, are designated by a number, and this number refers to the sample sent to the Bureau of Entomology for diagnosis, from which the organism was originally isolated. By referring to the card on file for such sample, various data concerning the original source of the culture are available.

BOUILLON.—Most of the bouillon used was prepared from beef, and such bouillon is more satisfactory than that from meat extract, provided a good quality of fresh meat is available. Bouillon was also prepared from Liebig's meat extract, using 0.3 gm. of the extract per 1,000 c. c. of water, adding 1 per cent of Witte's peptonum siccum and 0.5 per cent of sodium chlorid. Bouillon prepared from meat extract should be used only when good fresh meat can not be secured.

SUGAR-FREE BOUILLON.—The small amount of muscle sugar present in bouillon made from meat is eliminated by inoculating with *B. coli communis*. Fermentation tubes of bouillon prepared from Liebig's meat extract, when inoculated with *B. coli communis*, failed to yield gas and increased acidity; hence, such bouillon may be used as a base for sugar media without being treated with this organism. Dunham's peptone solution is also used sometimes instead of sugar-free bouillon in growing cultures for the indol test and in preparing the various sugar media. Its use is quite satisfactory in the study of the reaction changes from day to day, but is not satisfactory for the observation of growth characteristics.

ACID AND NEUTRAL BOUILLON.—Plain bouillon is made neutral or acid to the desired degree by adding the proper quantity of *N*/1 sodium hydroxid or *N*/1 hydrochloric acid, as determined by titration.

AGAR.—One and one-fourth to one and one-half per cent of agar-agar in plain bouillon is used. The appearance on agar plates of the members of the group studied constitutes an important differential point; hence, this is a valuable medium. Owing to the absence of muscle sugar in the bouillon prepared from Liebig's extract, it was found desirable to add about 0.1 per cent of glucose to such agar medium.

POTATO SLOPES.—The potatoes are thoroughly washed and peeled by removing generously the outer portions, after which cylinders are removed by means of a cork borer and cut diagonally. The pieces are washed in several changes of water, tubed with a liberal quantity of water, and sterilized. It is advantageous to leave a liberal quantity of water in the tubes on inoculation, otherwise characteristic growth is not apt to occur.

SERUM.—This medium is prepared from both calf's blood and that of the horse by heating them over a period of several days in a serum inspissator after the usual described methods.

POTATO WATER.—A few of the strains were inoculated into potato juice prepared by adding an equal amount of water by weight to very small pieces of potato, boiling, straining, and filtering the mixture, and then distributing into test tubes. Little value is attributed to this medium alone, since the growth does not differ from that on slopes with a liberal quantity of water.

MILK.—Owing to the difficulty of procuring separator milk, bottled milk, as purchased on the market, is used, first pipetting off the top cream, heating, setting aside overnight, again pipetting off any layer of

cream, and repeating until milk with a minimum of cream fat is secured, after which it is distributed into straight tubes.

LITMUS MILK AND AZOLITMIN MILK.—In the earlier studies an aqueous solution of ordinary litmus was used; later it was found that a solution of the pure blue dye azolitmin eliminated the objectionable feature of the red dye, which is present when litmus solution, as ordinarily prepared, is used (14, p. 419).

GELATIN.—Bouillon is used as a base to which gelatin to the amount of 10 per cent is added. The character of the growth, rate of liquefaction, form assumed by the liquefied area, degree and character of turbidity, membrane, and sediment were noted.

GLYCERIN GELATIN.—To gelatin prepared as noted above 5 per cent glycerin was added. The growth was studied and noted as for plain gelatin.

REDUCTION OF NITRATES TO NITRITES.—In testing for nitrate reduction, cultures were grown in a medium made up of Witte's peptonum siccum, 1 gm., and sodium nitrate, 0.2 gm., in 1,000 c. c. of tap water. To the culture grown in this medium and to the control tube, after five days' incubation, there were added equal parts of solutions A and B of Griess's reagent for nitrites as follows:

Solution A (Sulphanilic acid): 0.5 gm. is dissolved in 150 c. c. of 3 per cent acetic acid.

Solution B (Alpha-naphthylamin): 0.1 gm. is boiled with 20 c. c. of water and filtered hot through a small filter from which nitrites have been removed by washing. It is then diluted with 180 c. c. of 3 per cent acetic acid.

INDOL.—Cultures are incubated for 10 days in either sugar-free bouillon or Dunham's peptone solution; at the end of this period, to the culture and to the control tube are added first strong sulphuric acid, carefully poured down the side of the tube, followed in the same manner by a 0.02 per cent solution of sodium or potassium nitrite. A delicate pink ring forms at the juncture of the acid and culture in the presence of indol, or a pink coloration of the whole tube on agitation, if the coloration of the ring is of sufficient intensity.

ADDITIONAL NOTES ON TECHNIC

MORPHOLOGY AND MICROMETRY.

(*a*) VEGETATIVE FORM.—In both length and breadth these two organisms, *B. vulgatus* especially, vary not a little. This variation occurs not only in different strains, but in the same strain at different times and on different media. It is not unlikely that a number of conditions operate to produce such variation, such as amount of moisture, temperature, amount and rate of growth, and various other undetermined factors of development. In giving measurements, data concerning the medium, age of culture, and staining should be noted. Twenty-four-hour-old agar cultures were used for vegetative forms, and owing to the

greater difficulty of getting good stained preparations of spores measurements were taken from the best preparations obtainable, regardless of the age.

(*b*) SPORES.—At first it was thought that *B. mesentericus* with small spores was quite sharply marked off from *B. vulgatus* with relatively much larger spores. In general, strains of *B. mesentericus* have much smaller spores than those of *B. vulgatus;* yet there are strains of *B. mesentericus* that have comparatively large spores and strains of *B. vulgatus* which have comparatively small spores. Spores probably do not vary as much as do the vegetative forms.

FLAGELLA

In staining for flagella the technic employed by Williams, as presented in Mallory and Wright (10),[1] gave the best results.

MOTILITY.—No trouble is experienced in observing motility in any of the strains studied. Besides the ordinary hanging drop, the scheme of cutting small blocks from cultures growing on thin agar in petri dishes, and inverting just as in a hanging drop over a concavity in a slide, is very instructive in studying form and movement. The germination of spores may also be studied by this method. The small blocks can easily be made to adhere to the surface of the cover slip by applying a small amount of sterile bouillon.

STAINING.—Basic carbol-fuchsin is quite satisfactory for staining and is used almost exclusively. In staining by Gram's method, young 24-hour-old agar cultures are used and the stain is prepared fresh each time as follows: 5 per cent carbolic-acid solution, 10 parts; saturated alcoholic solution of gentian-violet, 1 part; allow the stain to remain on the thin smear for 5 minutes; without rinsing put in a watch glass and cover with

[1] Williams' method for staining flagella:

1. Flood the cover glass with a mordant consisting of:
   Alumnol 1% solution 5 c. c.
   Osmic acid 2% solution 5 c. c.
   Tannin 20% solution 5 c. c.
   Shake the mixture and add three drops of glacial acetic acid and shake again.
2. Apply the mordant less than one minute without heating. Wash thoroughly in water.
3. Cover the preparation during about one minute with a 1% solution of $AgNO_3$ to which sufficient ammonium hydroxide has been added to keep the silver in solution.
4. Wash in water.
5. Wash with 0.6% solution of sodium chloride.
6. Flood the preparation with a 30% solution of ammonium hydroxide and immediately wash in water.
7. Apply a few drops of Ortol photographic developer. The directions for making up this developer come with the Ortol.
8. Wash in water.
9. Cover with a 1% solution of gold chlorid during a few seconds.
10. Wash in water and apply Ortol developer for a few seconds.
11. Wash in water and cover with a 1% solution of mercuric chlorid for a few seconds.
12. Wash in water.
13. Apply Ortol developer for a few seconds.
14. Wash in water and repeat the application of chlorid of gold, the washing and the application of the developer two or more times.

One can substitute other developers for the Ortol with apparently as good success. A trial of the method will serve to convince that it is not so long or complicated as a perusal of the directions would indicate.

Lugol's solution, of a rich port-wine color, for 2 minutes, then again, without rinsing, put in 95 per cent alcohol for 15 minutes to decolorize.

OXYGEN REQUIREMENTS.—In determining oxygen requirements, cultures are inoculated into a deep tube of 1 per cent glucose agar after thorough boiling to drive off oxygen. The tubes are cooled as rapidly as possible after inoculation. The presence or absence of growth at the surface and in the depth of the tube is noted.

ANIMAL INOCULATION.—Rabbits and guinea pigs were used for testing the pathogenesis of the organisms. Neither *B. vulgatus* nor *B. mesentericus* was found to be pathogenic for guinea pigs. *B. vulgatus* was not found to be pathogenic for rabbits. Inoculations were made subcutaneously.

THERMAL DEATH POINT.—One will be impressed by the great discrepancy in the results of the experiments of different investigators to determine the amount of heat required to kill organisms or their spores. Rosenau (12, p. 683), in a study of the temperature required to kill the tubercle bacillus, gives a table showing the results gained by 32 investigators, including his own. The results of only 8 of these investigators agree. Discrepancies equally as great occur in the results given for organisms other than the tubercle bacillus, as will be found on consideration of the literature. Such lack of agreement in results may be only apparent. It is not at all unlikely that the temperature at which organisms or their spores are killed varies under different conditions more than is generally supposed. Among the factors which may operate to cause such a variation in results may be mentioned the age of the culture, the kind of medium used, including the amount of moisture present, the length of time of incubation, the amount of culture heated, etc. If the results secured are to be of the greatest practical value, an attempt should be made to determine under what conditions organisms or their spores withstanding the greatest amount of heat are produced and exist. In the attempts to determine the thermal death point of *B. vulgatus* and *B. mesentericus* two methods were used. In one a suspension of spores was prepared in test tubes of bouillon loosely plugged with cotton (13). The tubes were placed in an open vessel of water of the temperature desired, and, after time was allowed for the tubes to acquire the temperature of the water bath, the heating was continued for the desired length of time at the desired temperature. The tubes were then removed from the water bath and cooled rapidly. In the other method, tubes of soft glass of about ⅛ inch internal diameter were drawn to a capillary tube at one end and in the middle, leaving two bulblike expansions. These were sealed in the flame at one end after drawing up the spore suspension. Owing to the fact that one capillary end was left open during the heating, this method can not be used for the boiling temperature, since some of the suspension is forced out, contaminating the water bath and consequently the outside of the other tubes. By

the use of these thin tubes, the spore suspension acquires the temperature of the water bath almost immediately. In testing *B. vulgatus* and *B. mesentericus* for the thermal death point, spores of varying age were used. In the earlier experiments, where the heatings were made for 10 minutes at temperatures of 90° C. and under, no record was made of the age of the spores. In these early experiments heating at 90° for 10 minutes did not kill the spores, as a growth was secured in the tubes of spore suspension after heating and on agar plates made from the tubes. In the later experiments, cultures of various strains of *B. vulgatus* and *B. mesentericus* on potato and agar slopes about 1 month old were used. In such cultures there are practically only spores, and it is likely that at this age they have lost little of their vitality. The greatest amount of heat applied was 100° C. for 20 minutes, with the result that growth occurred in some of the tubes containing spore suspensions of *B. vulgatus* and on agar plates made from the tubes. *B. mesentericus* seems not to survive the amount of heat that some of the strains of *B. vulgatus* do. Beyond these statements the writer does not wish to commit himself. It is believed that much careful work, with spores of known age from different strains on different culture media, and secured under different conditions of growth and development, will be necessary to establish the maximum amount of heat required to kill the spores of the organisms studied.

Bacillus mesentericus (Flugge), Lehmann and Neumann (8), Chester (2)

Syn. *Bacillus mesentericus fuscus* Flugge.

OCCURRENCE.—Found everywhere about the apiary, on combs, and on different parts of the hive. Found on culturing larvæ, both diseased and healthy.

MORPHOLOGY AND MICROMETRY.—Hanging-drop and hanging-agar-block cultures show a finely granular organism occurring in ones and twos and short chains of a dozen or so individuals. Rarely coarse metachromatic granules may be seen. The flagella are distributed over the body. The rods are smaller than those of *B. vulgatus*, measuring from 1.3 to 2.8 μ in length and from 0.4 to 0.8 μ in width. The average length, however, lies nearer the mean of the extreme lengths observed above. Spores are usually formed in the middle of the rod, but may be found nearer one end. They measure from 1.0 to 1.9 μ in length and from 0.5 to 0.8 μ in width.

MOTILITY.—The organism is actively motile.

GRAM'S STAIN.—It appears that the organism takes Gram's stain only partially.

OXYGEN REQUIREMENTS.—Aerobic and facultatively anaerobic, probably growing better at the surface of the deep glucose-agar tube than throughout the medium.

AGAR COLONIES.—The colonies at the surface are circular and usually smaller than those of *B. vulgatus*, although they may spread over the

plate, and are gray, raised, and glistening. Under a power of 90 diameters they are found to be finely granular, more dense at the center, where a distinct nucleus is seen. At the periphery the colonies fade out to a very delicate stippled appearance, the margin entire and clear-cut without the pronounced outgrowths as in *B. vulgatus*, although they may show long finger-like radiating lobes; they are also unlike *B. vulgatus* in lacking the characteristic convolutions and mottlings. (Pl. 93, *A*). Deep colonies in the gross are gray and pinpoint-like. Under a power of 90 diameters the colonies are dense, quite irregular in form, and occasionally show slight mosslike branching.

GELATIN COLONIES.—Colonies are at first barely visible. Under power these colonies are of all shapes, from circular to the most bizarre types imaginable, often resembling cockleburs with rather unusually long projecting spines. Surface colonies are circular in outline, and liquefy, with the formation of a gray membrane which floats on the surface of the liquefied area.

BOUILLON.—The growth forms a heavy turbidity with a luxuriant growth around the wall of the tube at the surface which often does not until later close over at the surface of the liquid to form a complete cup-like membrane of luxuriant growth usually much resembling ground glass. The membrane usually turns yellow later. The turbidity increases and the medium remains often heavily turbid for days; later the turbidity may clear from the sinking of the organism and this, together with the sinking of the ground-glasslike membrane, which may be re-formed several times after sinking, produces a large amount of viscid sediment which may be made to arise from the bottom in the form of a long twisted spiral on agitation. A luxuriant ring growth is usually left around the wall of the tube at the surface. The above characteristics of bouillon growth apply also to the various sugars—namely, glucose, lactose, saccharose, levulose, maltose, and the alcohol mannite. As regards the reaction in bouillon and the various sugars and mannite, it may be said that there is no sharp definite reaction, especially for mannite and plain bouillon. Usually an acid reaction may be found at the fifth and tenth days in glucose, levulose, maltose, and lactose, when carefully titrated with *N/20* sodium-hydroxid solution. Plain bouillon, mannite, and saccharose are less likely to show a perceptible change in reaction. So variable are these reactions that it would not be feasible to submit a type series.

GAS PRODUCTION.—No gas is produced in fermentation tubes of glucose or in deep tubes of glucose agar.

SERUM.—There is usually some liquefaction, the resulting liquid being light to dark amber in color.

POTATO.—The growth varies in the same strain at different times and in the different strains. Usually, however, the growth presents a characteristic reticulated or netlike appearance. Often the growth above is smooth, glistening, raised, and butter-like. The color of the growth is

yellow, brown, or a dull slate. To secure the characteristic netlike growth, there must be an abundance of water in the butt of the tube (Pl. 94, *B*).

AGAR SLOPE.—A gray, raised, glistening growth spreads rapidly over the surface of the medium. Later the growth turns brown or yellow, and a gray ground-glass cuplike membrane often forms at the butt over the water of condensation.

MILK.—A coagulum is formed usually after about the third day. Following this, there is partial digestion of the coagulum, which is never complete, as in *B. vulgatus*. The coagulum may soften or undergo change in consistency and rise in the tube later, so that a very thick, slimy liquid results.

AZOLITMIN AND LITMUS MILK.—Sometimes a change may be noticed in 24 hours, the color turning slightly pink; later changes are manifest either by the tube turning a deep wine-red color or forming a soft, curdy, pink coagulum. As in plain milk, the curd is not entirely digested, a residue always remaining at the lower part of the tube, or the whole tube is a brown or black, thick, slimy liquid.

GELATIN STAB.—Liquefaction is not evident usually until the second or third day; then a slow, stratiform liquefaction proceeds, usually complete after a month or six weeks. A gray membrane is formed at the surface; and in the liquefied medium there is a turbidity, and floating flakes are present. Later the medium clears, forming a large amount of sediment at the bottom of the tube.

FIVE PER CENT GLYCERIN GELATIN STAB.—Growth is sometimes very slight without liquefaction. Liquefaction, when it takes place, is much slower than in plain gelatin, and is incomplete, but there are the same growth characteristics as to membrane, turbidity, and sediment as in plain gelatin.

INDOL.—None observed.

NITRATE REDUCTION TO NITRITE.—Usually negative. Sometimes a positive reaction may be observed in a strain which usually gives a negative reaction.

Bacillus vulgatus (Flugge), Migula, Lehmann and Neumann (8)

Syn. *Bacillus mesentericus vulgatus* Flugge; *Bacillus A*. White (16). Common name, potato bacillus

OCCURRENCE.—Same as that of *Bacillus mesentericus*.

GELATIN COLONIES.—Colonies at first are barely visible to the naked eye. Under a magnification of 90 diameters deep colonies are found to be more or less circular, irregular, finely granular, and often quite dense, with mosslike outgrowths, or with long raylike processes. Surface colonies present at first a finely granular appearance, and later usually become folded and mottled; later a membrane, more or less cotton-like, floats on the liquefied area.

AGAR COLONIES.—If only a few colonies are on a plate, they spread rapidly to almost any size, are not circular, and do not manifest a clear-

cut margin, as do the colonies of *B. mesentericus*. The appearance is characteristic under a power of 90 diameters, a marked folding resembling the convolution of intestinal coils being present. Sometimes this folding is less pronounced and the colonies present a peculiar mottled appearance, showing distinct denser and lighter areas of growth (Pl. 93, *B*).

MORPHOLOGY AND MICROMETRY.—In the hanging-drop or hanging agar block the organisms are quite distinctly granular, usually large metachromatic granules showing distinctly. The organism occurs singly, in pairs, and in long chains. The flagella are distributed over the body. The rods are quite variable in size, being 1.3 to 5.0 μ long and from 0.6 to 1.3 μ wide. Spores are usually formed in the middle of the rod, although at times spores are found nearer one end. They measure from 1.1 to 2.5 μ in length and from 0.7 to 1.3 μ in width.

MOTILITY.—The organism is rather actively motile.

GRAM'S STAIN.—The organism is stained by Gram's method.

OXYGEN REQUIREMENTS.—Aerobic and facultatively anaerobic with best growth at or near surface of the deep Liborius tube.

BOUILLON.—Growth varies in bouillon. Usually sooner or later a luxuriant gray, ground-glasslike, wrinkled membrane forms, extending for a considerable distance up the walls of the tube. The medium becomes uniformly turbid, increasing in degree for a time and later clearing, usually at the time the membrane forms. Later from the sinking of the membrane and clearing of the medium a large amount of viscid sediment forms rising from the bottom of the tube as a spiral on agitation. The membrane at the surface may sink and be re-formed many times. As the membrane sinks, there is usually left at the surface of the tube on the walls a ring growth. The membrane is apt to turn yellow or brown toward the fifth day and onward. The above characteristics of bouillon growth apply also to the various sugar bouillons—namely, glucose, lactose, saccharose, levulose, maltose, and the alcohol mannite, not different essentially from *B. mesentericus*, except that the membrane is usually more luxuriant, more wrinkled, and turns brown or yellow sooner. The membrane is not, however, so characteristically ground-glasslike, and the medium clears sooner. As regards the reaction in bouillon and the various sugars and mannite, what was said under *B. mesentericus* applies to this organism.

GAS PRODUCTION.—No gas is produced in fermentation tubes of glucose or in deep tubes of glucose agar.

SERUM.—Some liquefaction was noted in some of the strains. A luxuriant, gray, glistening, raised growth appears over the whole surface within 24 hours. Later, the growth turns yellow or brown as liquefaction proceeds and the resulting liquid varies from light to dark amber.

POTATO.—A luxuriant, moist, gray, raised, much-wrinkled growth takes place, most pronounced in the butt, especially over the water.

Sometimes the growth is yellow, brown, or pink, imparting the same coloration to the potato. A liberal amount of water must be present in the tube to secure the luxuriantly wrinkled and folded characteristic growth (Pl. 94, *A*).

AGAR SLOPE.—A luxuriant, gray, raised, glistening, moist growth spreads over the whole surface. Often the growth is more or less wrinkled. Later the coloration is brown or yellow, with occasionally a slight coloration of the medium near the surface.

MILK.—Most strains exhibit a firm coagulum within 24 hours, then the coagulum is gradually digested, the band of turbid whey gradually extending toward the bottom until the whole tube is a thick liquid. Often there are floating flakes or masses, and a viscid sediment.

AZOLITMIN AND LITMUS MILK.—Usually a pink coagulum is formed within 24 hours; later this is digested so that a gradually extending zone of turbid wine-red liquid is left above; still later the whole tube becomes a thick brown liquid with floating flakes or masses and a viscid sediment. At times no firm coagulum is noted; but the first change observed is the deep wine-red turbid liquid, which later turns brown. The changes in litmus milk show greater variation than in plain milk. The turbid brown coloration may appear early without the previous turbid wine-red coloration, or the brown liquid may be streaked with red or violet, or the tube may be a turbid brown liquid at first and later assume the red or violet coloration as a whole or in streaks.

GELATIN STAB.—Growth is usually manifest within 24 hours. Liquefaction proceeds rather rapidly, and is infundibuliform, as a rule, but usually becomes stratiform later. The medium is turbid, with a luxuriant membrane at the surface and growth extending up the side of the tube. Later the membrane sinks and the liquefied medium clears, forming a large amount of sediment, which is flaky or cotton-like in appearance.

FIVE PER CENT GLYCERIN GELATIN STAB.—Same as plain gelatin, except that the growth is slower and usually less luxuriant.

INDOL.—None observed in any of the strains.

NITRATE.—Reduction to nitrite is positive, with a strong red coloration on adding Greiss's reagent.

DESCRIPTION OF A STRAIN FROM SAMPLE 2329

In general, the cultural features and the morphology of the strain from sample 2329 resemble those of *B. vulgatus*, especially in the wrinkled and coiled growth on potato. On agar plates, however, this strain has never manifested the characteristic folding or mottling of *B. vulgatus* as seen under a power of 90 diameters, always presenting a nearly even homogeneous structure, with a strikingly clear-cut periphery, and usually as finely granular structure as in *B. mesentericus*. Moreover, there is not the same tendency to a spreading growth, the colonies usually being small and decidedly convex or pulvinate, and often on agar presenting a

decidedly yellow coloration. On gelatin the colonies are always of a pronounced yellow color. In the slowness with which it liquefies gelatin and coagulates milk and in its not reducing nitrates to nitrites, it resembles *B. mesentericus*. It is a large granular organism with metachromatic granules and has large spores, thus suggesting *B. vulgatus*.

Bacillus orpheus White

OCCURRENCE.—Encountered as an occasional secondary invader in larvæ infected with European foulbrood.

AGAR COLONIES.—Surface colonies small, often barely visible. Under a magnification of 90 diameters the colonies show a delicate, finely granular structure. Surface colonies may spread to any size, and a spreading growth may even cover the whole agar plate. Such growth is usually rather delicate and of a pale bluish tinge. Deep colonies are denser, irregular in form.

MORPHOLOGY.—In the hanging-drop the organism has a granular appearance, and is more slender than *B. vulgatus*. It occurs singly, in pairs, and in long chains, the division line separating individual organisms being difficult to make out. The ends of individual organisms are rounded markedly, appearing almost bluntly pointed. The spores are excentrically placed, and measure from 1.1 to 2.2 μ in length and from 0.6 to 1.2 μ in width.

MOTILITY.—The organism is rather actively motile.

GRAM'S STAIN.—The organism does not stain by Gram's method.

OXYGEN REQUIREMENTS.—Aerobic and facultatively anaerobic.

BOUILLON.—Frequently no growth of importance takes place until after 24 hours. The medium becomes turbid; often a luxuriant, gray, wrinkled membrane forms, and there are usually floating flakes. The medium is likely to clear later, with the formation of a considerable amount of sediment. The above characteristics of bouillon growth apply also to the various carbohydrate media—namely, glucose, lactose, saccharose, levulose, maltose, and mannite. On titration there are no definite reaction changes in the carbohydrate media.

GAS PRODUCTION.—No gas is produced in fermentation tubes of glucose bouillon or in deep tubes of glucose agar.

SERUM.—A luxuriant, raised, reticulated growth occurs. There is little, if any, liquefaction, and the water in the butt supports a gray membrane.

POTATO.—The growth on potato is slight and practically invisible.

AGAR SLOPE.—Within 24 hours separate areas of a delicate growth occur. Later the areas become confluent, and a luxuriant, gray, raised glistening growth appears over the whole surface.

MILK.—Milk is coagulated after several days. The coagulum is then slowly digested.

AZOLITMIN MILK.—The color is soon discharged to a dull pink or brown, coagulation taking place later. The coagulum is slowly digested.

GELATIN STAB.—Liquefaction proceeds slowly until complete. A gray membrane forms at the surface, and gray flakes float in the liquefied medium.

INDOL.—A very strong pink coloration takes place on adding the reagents.

NITRATE.—Reduction to nitrite was positive for one of the two strains tested and negative for the other.[1]

The description of *B. orpheus* is not as complete as it might be made. This is owing to the fact that it was only decided rather late in the course of preparation of the paper to include a description of *B. orpheus* at all.

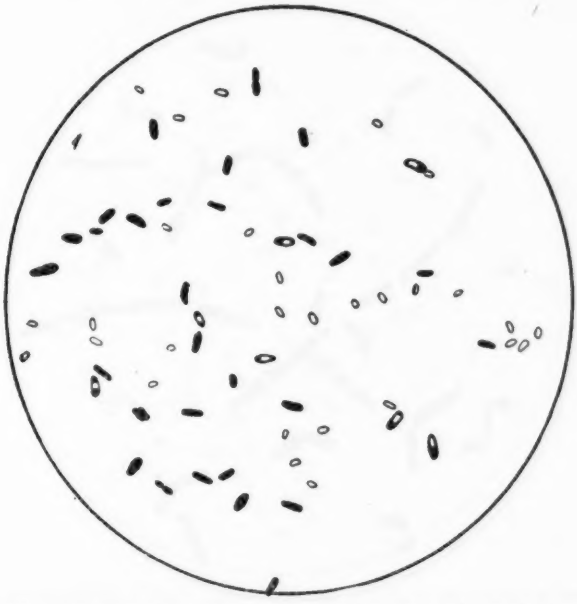

FIG. 1.—*Bacillus mesentericus:* Smear from an 18-hour-old agar slope.[2] × 1,480.

The description is based on the behavior of two different strains in the different media. White (18) discovered and named this organism in 1912.

The following brief characteristics should aid in the identification of these spore-forming organisms:

(*a*) *B. vulgatus* forms a heavy, wrinkled growth on potato (Pl. 94, *A*); *B. mesentericus*, a less luxuriant, netlike growth (Pl. 94, *B*).

(*b*) Even under low magnification, cultures on agar plates show that *B. vulgatus* (Pl. 93, *B*) grows in curves much resembling *B. anthrax*, while

[1] In old cultures of agar and plain milk a decided green coloration of the medium is noted.

[2] The drawings reproduced in figures 1 to 6 and Plate 93 were made with the aid of a camera lucida, the image being projected to the base of the microscope stand. The photograph of the potato cultures was made for the author by Dr. James A. Nelson, of the Bureau of Entomology.

colonies of *B. mesentericus* (Pl. 93, A) present a finely granular or stippled appearance.

(*c*) *B. alvei* and *B. orpheus* both grow slowly on potato; often it is almost impossible to detect any growth at all by the naked eye (Pl. 94, *C*, *D*), but stained smears must be made from the slope (fig. 3).

(*d*) The microscopic appearance of stained smears in the spore stage should be an important differential aid. Spores of *B. alvei* almost invariably show a vestige of the rod remaining (fig. 4); this does not occur in *B. vulgatus* (fig. 2), *B. mesentericus* (fig. 1), or *B. larvae* (fig. 5). The spores of *B. orpheus* (fig. 3) are distinctive, being excentrically placed.

FIG. 2.—*Bacillus vulgatus:* Smear from a 9-day-old agar culture. × 1,480. The rods are morer regular in younger cultures.

(*e*) *B. larvae* does not grow on the ordinary media of the laboratory, but requires media prepared from the juices of bee larvæ.

(*f*) Inasmuch as *B. pluton* occurs constantly and in such large numbers in European foulbrood larvæ, it was thought well to include a drawing of the organism (fig. 6).

PART II.—INOCULATION EXPERIMENTS

In the inoculation experiments not only the definitely different and typical species of *B. vulgatus* and *B. mesentericus* were used but also those atypical strains not partaking so strictly of specific biological characteristics. As will be seen from Table I, the inoculations include

the organism isolated from sample 2329, the strain of *B. vulgatus* secured from the Bureau of Animal Industry, and the strain of *B. vulgatus* isolated from potato.

MEDIA USED FOR GROWING THE CULTURES

Cultures were grown upon agar slopes prepared from both bee-larvæ bouillon and plain peptonized beef bouillon. The ordinary agar was prepared as usual by adding 1.25 to 1.50 per cent of shredded agar to beef bouillon titrated to +1.5 per cent to phenolphthalein. The bee-larvæ

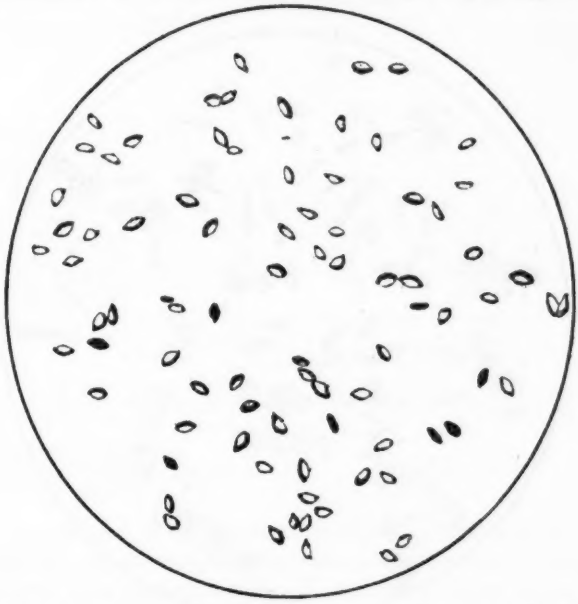

FIG. 3.—*Bacillus orpheus:* Smear from a 4-day-old agar culture. × 1,480. Note the excentrically placed spores.

agar was prepared by picking young bee larvæ from the combs, crushing, straining through cheesecloth, adding three times the volume of water, and then, as in the case of beef bouillon, heating in a water bath at a temperature of 60° to 65° C. for one hour. After filtering, peptone and sodium chlorid are added, and the reaction adjusted. To this, bee-larvæ bouillon titrated to +1.5 per cent to phenolphthalein, 1.25 to 1.50 per cent of agar is added.

AGE OF CULTURES AND METHOD OF INOCULATING

The age of the cultures at inoculation varies considerably. The usual method of securing cultures for inoculation is to sow the whole number of slopes designed for one colony at one time. Usually 30 slopes are fed

to a colony. These are given at the rate of 3 a day for 10 days. By this procedure the cultures at the last, or tenth inoculation, are 10 days older than those at the first inoculation, assuming that the three slopes were always given on every consecutive day without a break. The age of cultures at the first inoculation varies considerably, ranging from 1 day to 16 days. Sometimes the interval between inoculations for one or more times is greater than a day, thus advancing the age of the culture at the last inoculation considerably. Only one colony was inoculated with a fresh 24-hour-old culture at each feeding, since it was found that most cultures do not produce spores in abundance until somewhat older.

FIG. 4.—*Bacillus alvei:* Smear from a 48-hour-old agar culture. × 1,480. Note the vestige of rod clinging to nearly all of the spores. Note also the tendency of the spores to occur in rows.

While 3 slopes for 10 days was the usual allotment of a colony under inoculation, this was varied at times so that some colonies received a somewhat less number than 30 agar slopes of culture.

The cultures were inoculated by feeding to the bees in sterile sirup, washing the culture off of the slopes with sterile physiological salt solution by the use of a sterile glass rod. The cultures were fed when the organisms were forming spores in abundance.

DISCUSSION OF INOCULATIONS

Table I gives various data as to the number of colonies inoculated, kinds of media used, number of different strains, number of different

hives, seasonal range, and various other data by separate years, together with totals of same for the whole five years' inoculations.

TABLE I.—*Results of inoculation experiments with organisms of the Bacillus vulgatus group* [a]

Year.	Number of different strains.	Number of equivalent colonies fed cultures on—		Total inoculations equivalent to colonies.	Number of strains repeated—i. e., used on both bee-larvæ and ordinary agar.	Number of different colonies.	Seasonal inoculation range.	Number of slopes of agar.		
		Bee-larvæ agar.	Ordinary agar.					Bee-larvæ agar.	Plain.	Total.
1911.....	6 (2329, 2094, 2291, 2442, *B. vulgatus* B. A. I., bacillus from potato).	6	[b] 4	10	[b] 4	4	July 26–Sept. 16.	168	117	285
1912.....	4 (2904, 2905, 3309, and 3335).	[c] 2	4	6	[c] 2	6	June 28–Sept. 25.	60	118	178
1913.....	6 (3932, 3969, 4102, 4107, 4019, and 4016).	0	6	6	(*d*)	[e] 2	June 30–Sept. 27.	.....	174	174
1914.....	8 (4277, 4288, 4292, 4323, 4381, 4429, 4438, and 5356).	0	8	8	(*d*)	*f* 4	June 12–Oct. 5...	.....	218	218
1915.....	4 (4473, 4709, 4727, and 4823).	4	0	4	(*g*)	2	Aug. 2–Sept. 21..	107	.....	107
Total.	28.....	12	22	34	6	18	June 12–Oct. 5...	335	627	962

[a] Culture numbers refer to the laboratory numbers of samples of brood from which culture was obtained.
[b] 2329, 2094, 2291, and 2442.
[c] 2904 and 2905.
[d] Used only on ordinary agar.
[e] Three used also last year.
[f] One of these fed three cultures: 4288, 4323, and 4438.
[g] Used only on bee-larvæ agar.

In a consideration of the number of colonies inoculated it will be seen that a different colony was not always used for each strain. This was not necessary or even desirable, since, if one strain shows no pathogenic effects upon feeding to a colony, the same colony may be used over and over again, as often as convenient, for the inoculation of other strains. There were used, for the five years, 18 different colonies, which, with the repeated inoculations of some colonies, brings the equivalent number of colonies up to 34. Twenty-eight different strains of organisms representative of the *B. vulgatus* group were inoculated. Twelve of these cultures were grown on bee-larvæ agar and 16 on ordinary agar, as prepared from beef bouillon. Six of the 28 strains were grown upon both ordinary agar and bee-larvæ agar. The total number of slopes inoculated was 962, of which 335 were on bee-larvæ agar and 627 on ordinary agar. The seasonal range of the inoculation extends from June 12 to October 5, which is the seasonal range of the inoculations for the year 1914, as can be seen from Table I.

RECOVERY OF THE ORGANISMS INOCULATED

In order to determine the presence of the organisms in the inoculated colonies, cultures of the young brood were made at frequent intervals. This was done by gently removing the larvæ from the cells and first washing in five or six changes of sterile water for the purpose of removing the organisms present in the food surrounding the grubs as they lie in the cells. After this the larvæ were crushed on a sterile microscope slide, and a loop of the crushed contents was plated out on an agar plate. By the estimation of the number of organisms on the agar plate after 24 hours'

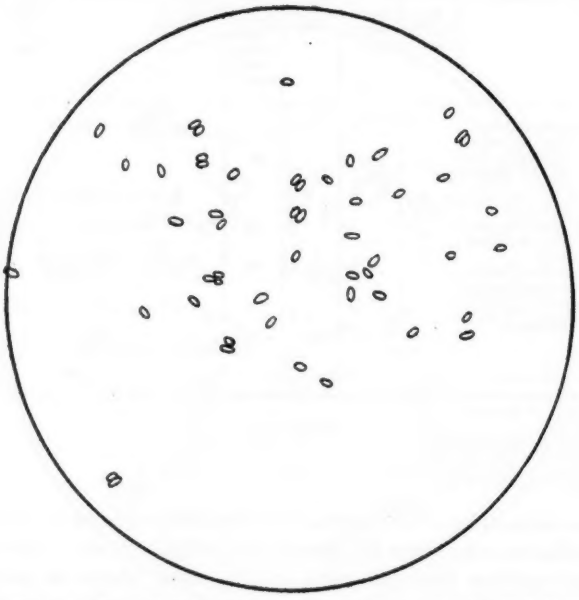

FIG. 5.—*Bacillus larvae:* Smear direct from a diseased larva. × 1,480. Note the small size of the spores as compared with those of *B. alvei*, the lack of any vestige of rod, and the lack of any tendency of the spores to occur in rows.

incubation, a fair idea is obtained of the number of organisms ingested. Of the 34 equivalent colonies inoculated, the organisms were in every instance recovered in abundance by culturing the larvæ. A loop of the crushed larvæ, when inoculated onto agar plates, often yielded colonies too numerous to count. The organisms were recovered in large numbers as early as the next day after the first inoculation. No exhaustive study was made to determine the length of time after the last inoculation that the organisms could be recovered, though in several instances they were cultured from the larvæ as late as eight months after; but the organisms were not in sufficient numbers to warrant the conclusion that their presence was due to the previous experimental inoculations. Nor-

mal larvæ were found to be nearly always sterile. This fact was pointed out by White in 1906 (16, p. 16, 29).

The writer has found occasionally a few organisms, both of *B. vulgatus* and *B. mesentericus*, on culturing apparently healthy larvæ, and on still rarer occasions a very large number in a single larva. This should excite no surprise when the great abundance of both of these organisms in nature, especially in water, is considered. Another method of determining the presence of the bacteria in the larvæ is by the study of paraffin sections. Larvæ were not sectioned from every colony inoculated, but enough sections were secured to show that the organisms were present in great

FIG. 6.—*Bacillus pluton:* Smear direct from a diseased larva. X1,480. Note that the organism stains uniformly, this distinguishing it from any spore-forming bacterium of the apiary.

abundance in the alimentary canal of the larvæ. It was hoped by sectioning larvæ and pupæ, as well as the shed skins and detritus in the bottom of the cell, that the ultimate fate of the bacteria in the gut of the bee might be learned. The results of such investigations are not definite enough for publication at this time. Indications point to the elimination of the bacteria some time after the brood passes to the pupal stage, since so comparatively few organisms were ever recovered from culturing the contents of pupæ, especially later pupal stages.

RESULTS OF THE INOCULATIONS

In none of the colonies inoculated over the whole five seasons of feeding of the cultures was there any appearance of the brood simulating either

American foulbrood or European foulbrood; nor were any other abnormal conditions detected. If we judge from the amount of culture or virus required to produce disease generally, more than enough of the culture was fed to produce disease. Also, the seasonal range is certainly sufficient to cover the time of appearance of any of the known infectious brood diseases of bees as occurring naturally, or by inoculation with the proper etiological factor. All of the known infectious brood diseases of bees—American foulbrood (17), European foulbrood (18), and sacbrood (19)—have been thus produced experimentally. Unfortunately Lambotte (7) does not describe sufficiently the method of preparing his agar medium from the juices of bee larvæ, and a medium might be devised to produce the variation claimed—that is, transformation of *B. mesentericus vulgatus* to *B. alvei*. But, if it is granted that Lambotte fed cultures of *B. alvei*, his results would not agree with present-day investigations, since *B. alvei* has been found not to produce disease upon repeated inoculation. Mindful of the variation possible in different strains of the same organism from a study of the *B. vulgatus* group, it seems, nevertheless, that the differences in biological characteristics of *B. alvei*, *B. vulgatus*, and *B. mesentericus* are too great to warrant expecting any transformation of one to the other in any short period of time.

SUMMARY

(1) Lambotte (7), in 1902, claimed to have produced "foulbrood" of bees by feeding them cultures of *B. mesentericus vulgatus*.

(2) Lambotte, in explanation of the above, states that *B. alvei* arises as a special variety of *B. mesentericus vulgatus* when the cultures are grown upon media prepared from the juices of bee larvæ.

(3) There are at least five spore-forming bacteria occurring commonly about the apiary—*B. vulgatus*, *B. mesentericus*, *B. orpheus*, *B. alvei*, and *B. larvae*.

(4) Mistakes in the identification of the above-mentioned organisms might well be made, especially without knowledge of their occurrence.

(5) There seems no doubt that many of the earlier investigators of bee diseases confused the identity of the spore-forming bacteria of the apiary.

(6) From our present knowledge of these organisms, their identification should be a matter of less difficulty.

(7) The author, from his study of *B. vulgatus*, *B. mesentericus*, and *B. alvei*, believes that the biological differences of the three species are too great to warrant expecting the transformation of one to another in any short period of time.

(8) Even if it is granted that Lambotte fed pure cultures of *B. alvei*, his results would not agree with those of present-day investigators of bee diseases, since *B. alvei* has not been found to produce disease in bees upon repeated inoculations.

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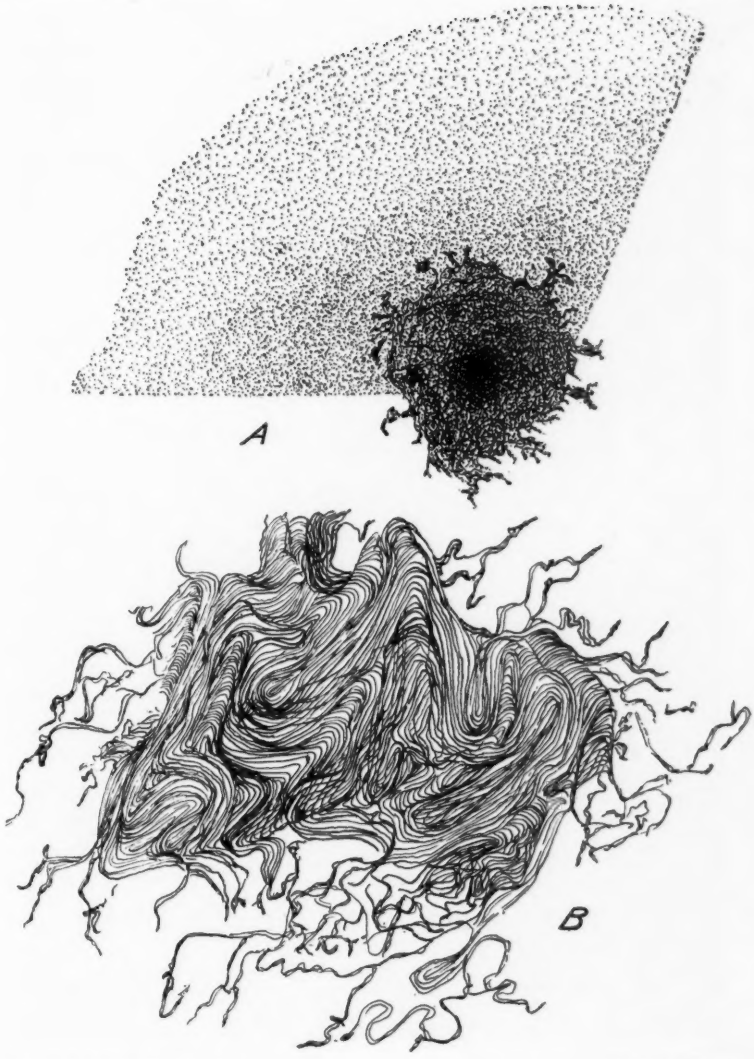
PLATE 93

A.—*Bacillus mesentericus:* Growth of a 24-hour-old surface colony on an agar plate. × 140.

B.—*Bacillus vulgatus:* Growth of a 24-hour-old surface colony on an agar plate. × 140.


A
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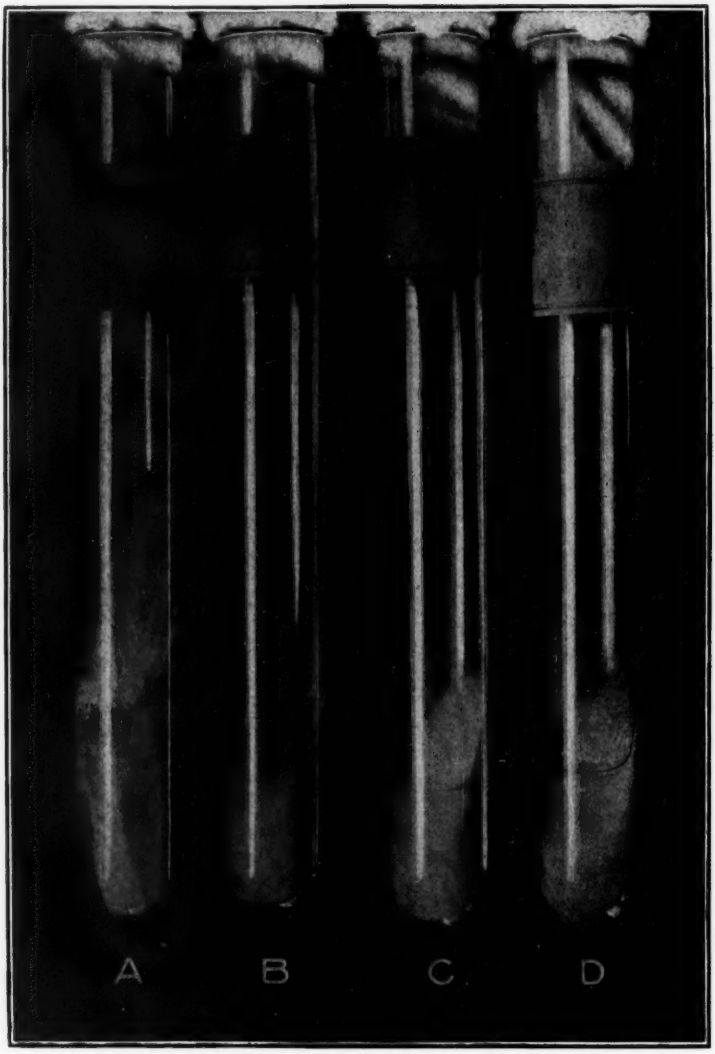
A
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C
D

PLATE 94

Growth of 24-hour-old cultures on potato slopes. All tubes contain a liberal quantity of water in the butt.

A.—*Bacillus vulgatus:* Note the luxuriant folded and wrinkled growth, especially on the surface of the water in the butt, and on the lower portion of the slope.

B.—*Bacillus mesentericus:* Note the less luxuriant growth. The growth on the surface of the water is more in the nature of a thin pellicle without any folding or wrinkling. Often a netlike appearance of the growth is noted.

C.—*Bacillus alvei:* No visible growth.

D.—*Bacillus orpheus:* No visible growth.

FUSARIUM-BLIGHT, OR WILT DISEASE, OF THE SOYBEAN[1]

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INTRODUCTION

During the summer of 1915 a package of diseased plants of the soybean (*Soja max* (L.) Piper (17);[3] syn. *Glycine soja*, *Soja hispida*, etc.)[4] was received from a correspondent at Red Springs, N. C. A large number of plants in the field from which these specimens were taken had become chlorotic, or were dead. The plants received were still green and in good condition for examination. The evidence obtained from a preliminary inspection indicated that the diseased condition was due to the presence of a fungus belonging to the genus Fusarium. Furthermore, nearly all of the isolations from this material gave pure cultures of a species of Fusarium.

The studies herein reported were therefore undertaken (1) to determine the parasitism of this species of Fusarium on soybean, (2) to establish its relationship to the Fusaria of the section Elegans in so far as a comparison of the cultural characteristics permit, and (3) by means of cross inoculations and field studies to determine the relationship of this disease of soybeans to the wilt disease of cowpeas (*Vigna sinensis* Hassk.) caused by *Fusarium tracheiphilum* Smith.

ECONOMIC IMPORTANCE OF THE SOYBEAN

The soybean is a native of tropical Africa, Asia, and Australia (23, p. 360–361; 17, p. 76) and was introduced into Europe by Kämpfer about 1690 (18, p. 9). At the present time it is the most important legume grown in Japan, China, and Manchuria. Its culture in England was begun in 1790. The plant was introduced into the United States from Japan in 1860. Since that time its cultivation as a soil-improving and a forage crop has been confined for the most part to the Southern States. North Carolina is probably foremost among these States in the production of soybeans. The yield in 1909 was 13,313 bushels,[5] and in 1915 was estimated[6] as approximately

[1] Published with the permission of the Director of the North Carolina Agricultural Experiment Station.

[2] The writer wishes to express his deep appreciation to Prof. H. R. Fulton, under whose direction the study was begun, and to Dr. F. A. Wolf, for his helpful suggestions and criticisms during the major part of the investigations and for aid in the preparation of the manuscript.

[3] Reference is made by number to "Literature cited," p. 438–439.

[4] For a complete synonymy, see Piper (17).

[5] U. S. Bur. Census, 13th Census, 1910, 1913. Statistics for North Carolina, p. 632.

[6] Estimate furnished by the North Carolina Experiment Station.

1,000,000 bushels. Within the last two or three years this crop has become increasingly important because of the variety of products manufactured from the oil and meal.[1] During 1915, $9,000,000 worth of oil alone was imported. Local cottonseed-oil mill owners have been induced, however, partially by the efforts of the North Carolina Experiment Station, to crush soybeans during their otherwise idle season. The few mills in the State which have done this have found a ready market for the oil and meal.

OTHER SOYBEAN DISEASES

Soybeans are very generally observed to be quite free from disease, and no very seriously destructive parasites of this host appear to have been reported in the literature at hand. Of those reported, a detailed study has not been made, except in the case of *Bacillus lathyri* Manns and Taubenhaus (13, 14). The accounts of the others consist of brief fragmentary mycological notes and mention of their place of collection or of their appearance. Since any of them may appear on plants affected with blight or wilt, it is deemed advisable to call attention to the published accounts of these diseases and the appropriate bibliography.

Septoria sojina v. Thûmen (on living or declining leaves) (24).
Phyllosticta sojaecola Massalongo (15, p. 688).
Aecidium glycines P. Henn. (6, p. 52).
Uromyces sojae (P. Henn.) Sydow (22, p. 429).
Bacillus sp. (on leaves)—Heald (9, 10), Smith (21), and Clinton (4).
Bacillus lathyri Manns and Taubenhaus (on leaves and pods) (13, 14), and Manns.
Heterodera radiciola—Scofield (19, p. 9), Gilbert (8, p. 9), Bessey and Byars (2, p. 8). (These authors merely mention the soybean as a host for this parasite.)
Chlorosis and crinkling (cause?) (Description of the disease in the field)—Clinton (5).
Septoria glycines T. Hemmi (comparison with *S. sojina* above) (11).

It is not believed that the presence of any of these organisms would lead to confusion in the diagnosis of blight caused by the species of Fusarium under consideration.

HISTORY AND OCCURRENCE OF THE DISEASE

No published report of a disease of soybeans caused by any species of Fusarium and one account only of attempts to produce a disease of this host with the cowpea wilt organism have been brought to the writer's attention. Orton (16, p. 16–19) conducted these tests at Edisto Island, S. C., in 1900, and at Monetta, S. C., in 1901. Several varieties of cowpeas and soybeans were planted on soil badly infected with the

[1] The following is a list of the most important products obtained from soybeans or in which soybeans enter: Soybean milk, meal or flour, soups, pork and beans, meat substitutes, fertilizer and cattle feed from the meal, and dynamite and high explosives, soaps, linoleum, rubber substitutes, margarine, Japanese sauce, paints, varnishes, toilet powder, waterproof cloth, salad oil, lubricants, and lard substitutes from the oil.

cowpea-wilt organism and with a nematode (*Heterodera radicicola*). Concerning the work at Monetta, S. C., he says (p. 18):

> Eight varieties [soybeans] were tried on ten plats. All proved to be immune to the wilt disease, but none of them was adapted to the local conditions. The growth was very small, the plants averaging from 8 to 14 inches high, though most of the varieties bore a good crop of seed for such small plants. All suffered from much drought in midsummer and all were badly injured by the root nematode. On examination of the roots a moderate number of bacterial tubercles were found. * * * They [soybeans] were at a considerable disadvantage in this test on account of the late date of planting and the ensuing dry weather.

The varieties tested were Tokio, Buckshot, Yosho, Ito San, Manhattan, Guelph, and Amherst. Orton reported that at Edisto Island the soybean made a heavy growth, 3 or 4 feet high and was free from the wilt disease. It may be said that a very considerable proportion of the several varieties of cowpeas grown in adjacent plots succumbed to wilt. The results of these tests accord with the observations of others who have had opportunity to observe these crops when they were grown on soil known to be infected with cowpea wilt.

A limited number of careful observations have therefore been made during 1915 and 1916 to determine whether the wilts of these two hosts are coextensive in range and thus to furnish evidence of the identity of the two. Two 5-acre fields on widely separated parts of the North Carolina Experiment Station farm, in which cowpeas and soybeans were grown in alternate rows, showed a very considerable proportion of the former host affected, whereas the latter remained entirely free from disease. In other localities of the State, soybeans growing on soil infected with the cowpea-wilt organism have remained disease-free.

Observations differing from these were made in the case of soil brought from another part of the Station farm. When this soil was used to grow soybeans in pots out of doors, it was found to be infected with soybean-blight, as shown by the development of the disease in 33 of the 80 pots. Wilt, both of cowpeas and soybeans, was present on the farm of the correspondent previously referred to, at Red Springs, N. C. Many of the soybean plants in this field were killed and many only stunted, so that a decrease in yield of 60 per cent during the past season is probably a correct approximation of his loss. Blight or wilt of soybeans has also been found to occur at Exum and Belhaven, N. C., and was the cause of considerable loss in both locations.

Since cowpea-wilt has been found in many localities throughout the Piedmont and the Coastal Plain sections of North Carolina, it is entirely probable, if we judge from the results to be presented subsequently, that the soybean-blight may appear more or less generally wherever the soil is infected with *Fusarium tracheiphilum*. Records received from the Office of Plant Disease Survey show that, up to the close of 1915, *F. trachei-*

philum has been reported as being productive of losses to cowpeas ranging from 2 to 100 per cent in Indiana, Missouri, Mississippi, Louisiana, Texas, Oklahoma, Georgia, Florida, North Carolina, South Carolina, and Virginia.

APPEARANCE OF THE BLIGHT IN THE FIELD

In 1916, soybeans were planted during the last two weeks in May. This is somewhat later than usual, being due to the late season and a period of drought. When the plants were 4 weeks old, they had attained a height of 2 to 3 dm. and were apparently still free from disease. The disease was first observed on July 25, when the affected plants were about 8 weeks old. Symptoms of the trouble could probably have been found a week or two earlier. Affected plants, all of the same age but varying in height from 2 dm. to 1 meter, were observed on the 25th. The fungus is believed to have stunted these small plants. In no case has the disease been observed on seedlings.

The contrast in appearance of five healthy and five diseased plants is shown in Plate 95, *D*, *E*. The same type of clay soil was used in both pots, and the plants in each were grown out of doors under the same conditions. The plants shown in figure *E* were naturally infected from the soil. A considerable number of the leaves have fallen from the diseased plants, a portion of the petioles persist, the plants are dwarfed, and there is no evidence of wilting in any part of the plants. The foliage which persists on these plants is yellow as contrasted with the normal leaf green of healthy plants.

The occasional absence of a definite wilting of the leaves has been noted in other wilt diseases. Orton (16, p. 10), in speaking of the cowpea disease caused by *F. tracheiphilum*, says:

> The term "wilt" is somewhat misleading, as the leaves usually drop off before there is any conspicuous wilting. The name was applied because of its relationship to the wilt of cotton and watermelons, where this symptom is very prominent, and it seemed desirable to retain it for the cowpea disease.

In the case of the soybean disease, wilting is a less prominent symptom than in cowpeas, and is very seldom present at any stage of the disease. The plants, as a rule, drop all of their leaves and die without any evidence of wilting. Wilting has been observed in a very few instances in the field in the case of young plants. The woody nature of the stem and petioles probably accounts for the general absence of wilting in them, and the presence of well-developed mechanical tissues in the leaflets may account for their failure to manifest wilt. The possibility exists, also, that the physiological interaction of parasite and host differs from that exhibited by wilted cotton and watermelons infected with *Fusarium* spp.

Instead of applying the name "wilt," therefore, to the soybean trouble, it is perhaps desirable to call it "blight or wilt," the former

word describing the most prominent symptom on the foliage and the latter retaining the idea of its relationship to other wilt diseases produced by species of Fusarium.

Soybean blight or wilt may make its appearance on individual plants, but does not cause the death of all the plants within definite areas, as in the case of cotton wilt.

Although no definite effort has been made to determine the method of entrance of the organism into the host, it is thought that it enters through the smaller roots in practically the same manner as that described for other diseases of this character. Many of the fibrous roots are destroyed, and new roots are formed of insufficient number, however, to maintain the life of the plant.

Perhaps the most prominent symptom is a browning or blackening of the interior of the stems and roots. As soon as the leaflets begin to drop, this discoloration is evident when the root and stem are split longitudinally. This character is shown in Plate 95, figures *B* and *C*, showing healthy and diseased stems, respectively. As the disease progresses, the discoloration extends upward in the stem for one-half or more of its length. The tracheal tubes of affected stems when cut obliquely show as brown spots. The relative amount of discoloration in general and the depth of color in affected xylem portions is less in soybeans than in cowpeas.

A large number of stained free-hand sections were made of stems at all stages of development of the disease. In the early stage, only the xylem tubes nearest the pith were found to contain the fungus filaments. The pith had disappeared in both normal and diseased plants of moderate size. Later, other of these tubes throughout the xylem area are penetrated and become filled to a large extent with a network of fungus filaments. In still more advanced stages, all of the xylem elements (fig. 1, *A–G*) were found to contain the fungus; and, in addition, the cortical parenchyma was invaded.

The surface of stems of plants in advanced stages of the disease generally have salmon-colored spore masses, sporodochia, thickly and irregularly distributed over them.[1] This character is shown by the roughened appearance of the stem in Plate 95, *A*. The spore masses are composed of macroconidia of the fungus and are frequently found to occur on plants whose upper leaves are still healthy in appearance. Sometimes they are formed only in more advanced stages of the disease.

MANNER OF INFECTION AND SPREAD OF THE DISEASE

As stated above, the fungus may enter the plant through the small roots. In addition to the spread of the organism through the soil, spores are so abundantly produced that drainage water, implements,

[1] Sporodochia on stems of cowpeas are reported by Orton (16, p. 9) to appear after the death of the plants.

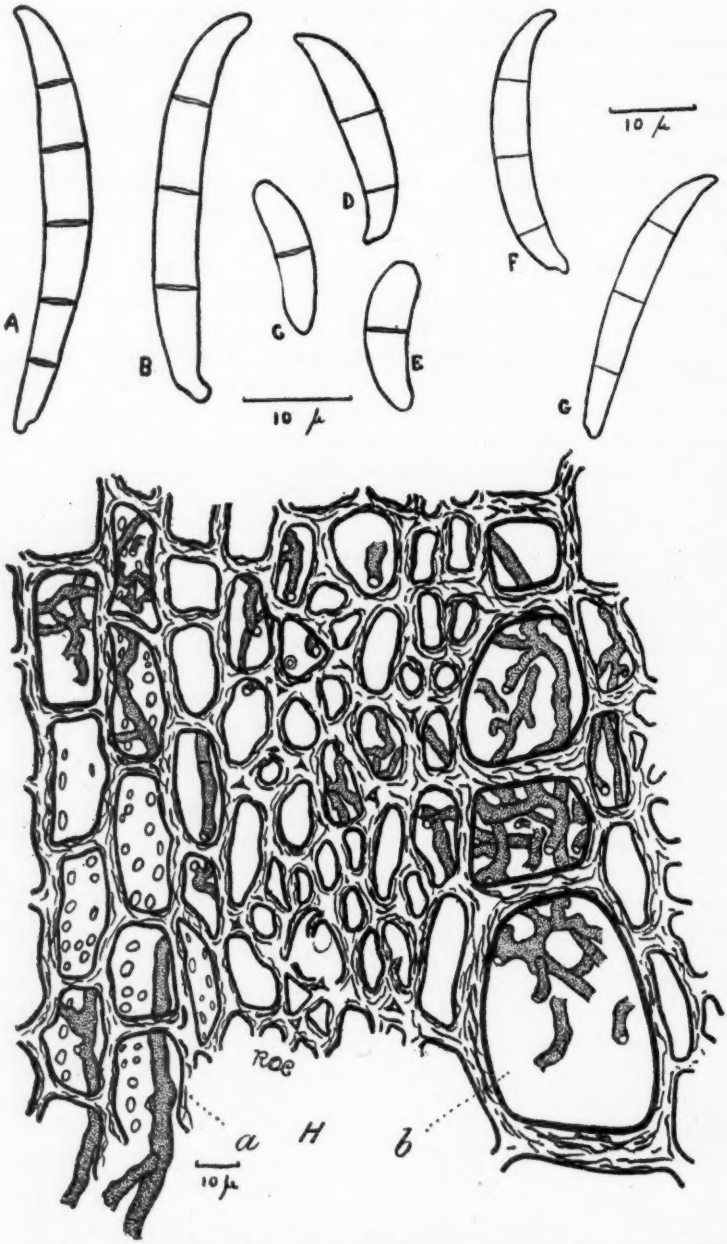

FIG. 1.—*A–G*, Types of macroconidia of the species of Fusarium on soybean. *H*, Cross section of the xylem portion of a diseased soybean stem, showing the invasion of the medullary rays (*a*) and the xylem vessels (*b*) by mycelia of the species of Fusarium on soybean.

and animals are also probably concerned in its spread. In all cases observed except one, nematodes have been present and probably facilitated the entrance of the fungus. The nematodes were found in infected sandy soil, but not in infected clay soil.

COMPARISON OF THE SOYBEAN SPECIES OF FUSARIUM WITH OTHER WILT-PRODUCING SPECIES OF THE GENUS

SOURCE OF CULTURES AND METHODS OF ISOLATION

Isolations were made from the interior of stems of freshly wilted soybean and cowpea plants. The stems were first thoroughly washed in water and allowed to remain wrapped in cotton moistened with 0.1 per cent solution of mercuric chlorid for 15 minutes. They were then split open so that the diseased interior was exposed. Fragments of diseased tissue were removed with a sterile scalpel and transferred to cooled poured plates of string-bean agar (8 c. c. per plate), to each of which four drops of 20 per cent lactic acid had been added. After several days, a microscopic examination was made of the conidia and mycelium to determine whether other organisms were present. Eight transfers to test-tube slants were made from the margin of several plantings and kept for comparison and for indications of contamination. It may be noted that a large percentage of pure cultures was obtained by this method. From the cultures that were pure, single-spore cultures were obtained according to the method described by Sherbakoff (20, p. 102–103; p. 104, footnote 8). Stock cultures were made from these single-spore cultures and repeatedly repoured to protect from subsequent contamination.

Several species of Fusarium were secured, in order to compare them with the *Fusarium* sp. from the soybean and the one from the cowpea, isolated as described above. The following species, subcultures from Wollenweber's authentic cultures, were obtained through the courtesy of Mr. C. W. Carpenter, of the Bureau of Plant Industry: *Fusarium oxysporum* (Schlecht.), *F. vasinfectum* (Atk.), *F. lycopersici* Sacc., *F. niveum* Smith (members of the section Elegans), and *F. discolor*, var. *sulphureum* (Schlecht.) App. and Wollenw. (1, p. 115–118), (section Discolor).

These species were studied in culture, in order to determine their morphological and cultural characters, since such a study is considered of primary importance in their differentiation. The species mentioned were chosen because all except one belong to the section Elegans, the section which contains the known wilt-producing species, and because, according to Wollenweber, they are the most difficult to separate by this method. *F. conglutinans* Wollenw., *F. redolens* Wollenw., and *F. orthoceras* App. and Wollenw., of the same section, are included in the comparisons. They are so different from the others, as indicated by the

original descriptions, that the writer soon realized that there was little probability of confusing them with the soybean strain. Wollenweber (25, 26) and Sherbakoff (20) have described other species and varieties of the section Elegans which are not, however, included in this study, because they occur on hosts widely separated genetically from the soybean [1] and because the authors have not had opportunity to make a sufficient number of infection experiments to establish them as wilt producers.

CULTURE MEDIA AND THEIR VARIOUS EFFECTS ON SPECIES OF FUSARIUM

In making a cultural study of these fungi much care was taken to follow the suggestions of Appel and Wollenweber (1), Wollenweber (25, 26), and Sherbakoff (20), in order to determine what criteria to employ in judging normal growth characters. It is generally believed that standardization of cultural methods is highly essential in the comparative study of so difficult a group of fungi.

The writer has kept the soybean and the cowpea strains under constant observation for two years on various kinds of "natural and artificial media" and under widely variable physical conditions. He is therefore familiar with the possible variability of members of this genus.

Since a large number of the media used did not prove to be of special diagnostic value, they are not discussed here. Among the media most commonly employed and serving some particular purpose were oat, potato, and string-bean hard agars (3 per cent agar), which, because of the paucity of moisture (20, p. 106), give all forms of fruitification with "normal" spores. Five to 10 per cent of dextrose was added to agars to favor the production of pigment. Growth on steamed rice in test tubes from weighed quantities of rice and measured amounts of water to obtain uniformity also results in the formation of pigment and sometimes an odor that is typical for certain related species of Fusarium. Herbaceous and woody stems, string-bean pods, and potato plugs give the best development of sporodochia and pionnotes.[2] Potato plugs also serve for the proper development of sclerotia and colors, both of which may be reduced or absent from stem plugs when there is a minimum development of mycelium.

According to Wollenweber (25, p. 37), virulence is commonly maintained on stem plugs. Living sterile soybean and cowpea seedlings grown in 6-inch test tubes were also used and are thought to be a better medium for maintaining virulence in the strains from the respective hosts.

[1] Wollenweber, H. W. (25, p. 37) says, "The parasite from one host, as a rule, has not been found on living organs of another host. In pure culture the parasite from one host . . . did not cause wilt in any other host as a result of inoculation experiments."

[2] For a discussion of these terms, see Wollenweber (25, p. 24, footnote).

In order to obtain sterile seedlings for this purpose the seeds were first washed for 5 minutes in tepid water and were then placed in concentrated sulphuric acid for 20 minutes. Formalin, mercuric chlorid, both in aqueous and alcoholic solution, and other disinfectants were employed with much less success. After washing off the acid in three or four changes of sterile water, the seed were transferred into sterilized moist chambers in the bottoms of which several layers of moist filter paper had been placed. Germinated seeds on which there was no evidence of contamination after a day or two were transferred to sterile test tubes [1] the bottom of each of which contained a wad of moistened filter paper.[2] If, during germination or transfer, contamination occurs, it generally becomes evident on the seedlings or white paper, especially if the seedlings are set aside until they have grown to a height of 3 or 4 inches.[3]

METHODS OF STUDY AND PRESENTATION

All transfers of different strains in a set for comparison were made to a certain medium on the same day and to additional media on later days until the set was growing on a sufficient number of media to provide the necessary cultural characters. When species were compared, they were always of the same age and were grown on the same medium. As many comparisons could be made on the same day as there were species and kinds of media in the set. If sufficient data had not been obtained, if certain cultures were abnormal, or if other species or media were to be used, new sets were prepared of all of the species using the desired media and comparisons were again made throughout the series.

Cultural differences also arise as a result of the employment of spores or a bit of mycelium in inoculation. In the former case the young cultures quickly produce spores with a scant mycelial growth, while in the latter the mycelial growth is abundant and there is a paucity of spores. For this reason spores from sporodochia, when present, were used, and in all cases, in so far as was possible, the same kind of inoculum was transferred for all cultures of a set. When the production of spores becomes subnormal, as it often does in cultures, considerable time and patience may be required to bring the strain back to a "*Normkultur*." This was accomplished by transferring a small portion of mycelium to a variety of media until a medium was found on which spores were again obtained.

[1] For making this last transfer, dip the ends of long tweezers into 95 per cent alcohol and ignite in the flame. This sterilizes instruments, burns off the excess of alcohol, and leaves them dry and cool enough for immediate use.

[2] The use of agar as a substratum for this purpose (Garman and Didlake, 7), and Sphagnum moss, did not prove to be satisfactory. Soil, too, has a disadvantage in that it does not show the contaminations as readily as filter paper or agar.

[3] An oat sprouter with glass front, heated by a kerosene lamp and costing about $10, makes a good light incubator for such purposes when the greenhouse is not conveniently located or the temperature suitable. This sprouter is unsuited, of course, to cultures or material requiring a constant temperature.

All cultures were kept in the laboratory at room temperature, 12° to 26° C., and in diffused daylight, so that they were subjected alike to any change of environmental conditions.

In all cases 10 cultures of a species were made on each medium. Different forms of fructification which normally appear on a certain medium may not do so in every tube. For example, in a species where sporodochia are not abundant, they may perhaps form on only 2 or 3 of the 10 stem plugs; or if the form produces green sclerotia, they may develop on not more than 5 of the 10 potato plugs. In some instances as many as 8 to 10 sets of 10 tubes each of a particular species were made.

In making the microscopical examination note was taken of the size, abundance, and type of conidia (fig. 1, *A-G*), chlamydospores, and conidiophores. In measuring spores several fields were first examined to fix in mind the prevailing type and an average of 10 or more of these typical spores was made. Careful note was taken also of extreme types.

In the macroscopic study of the cultures the nature of the stromata, the pionnotes and sporodochia, the character of the aerial mycelium, the color of spore masses, aerial and submerged mycelium and substratum, and the production of sclerotia were considered.

RESULTS OF THE COMPARISON OF THE SOYBEAN FUNGUS WITH OTHER MEMBERS OF THE SECTION ELEGANS

The first sets of parallel cultures were intended to serve in the separation of any or all of the species of Fusarium causing wilt from the soybean fungus. *F. discolor* var. *sulphureum*, *F. oxysporum*, *F. vasinfectum*, *F. lycopersici*, *F. niveum*, *F. tracheiphilum*, and *Fusarium* sp. from soybean were therefore grown on the following media, several sets of 10 cultures of each species being used on each medium: Potato plugs, steamed rice, cotton stems, potato hard agar, and string-bean hard agar. The cultures were examined when 8, 15, 19, 30, and 50 days old. The results are noted in Table I. Only those characters are recorded that are necessary or useful for the separation of the species.

TABLE I.—*Characters which separate a number of the wilt-producing species of Fusarium from F. tracheiphilum and the soybean fungus*

Species.	Sclerotia.	Sporodochia.	Pionnotes.	Chlamydospores.
F. discolor	None	Numerous	Perfect	Intercalary; no measurements.
F. vasinfectum	Green and flesh-colored.	do	do	Intercalary and terminal; no measurements.
F. oxysporum	do	Few	Reduced	Intercalary and terminal; 6 to 12 μ.
F. lycopersici	Flesh-colored	Numerous	Perfect	Intercalary and terminal; no measurements.
F. niveum	Large green	do	Reduced	Same as for *F. lycopersici*.
F. tracheiphilum	Green and flesh-colored.	Few	None	Intercalary and terminal; 6 to 12 μ.
Fusarium sp. on soybean	Mostly green; some flesh-colored.	do	do	Same as for *F. tracheiphilum*.

TABLE I.—*Characters which separate a number of the wilt-producing species of Fusarium from F. tracheiphilum and the soybean fungus*—Continued

Species.	Macroconidia.		Odor.
	Size of 3-septate.	Type.	
F. discolor	No data.	Discolor; mostly 5-septate.	None.
F. vasinfectum	Same as in *F. oxysporum*.	Elegans; mostly 3-septate.	Strong lilac on rice.
F. oxysporum	28.7 to 35.6 by 3.6 to 4.1 μ.	do.	Often none, sometimes scant lilac.
F. lycopersici	Abnormal.	do.	None.
F. niveum	Abnormal; (original description gives larger than in *F. Oxysporum*).	do.	Do.
F. tracheiphilum	23.6 to 41.0 by 3.9 to 4.1 μ.	do.	Do.
Fusarium sp. on soybean	24.6 to 35.8 by 2.89 to 4.1 μ.	do.	Do.

From the data in Table I it is important to observe that *F. tracheiphilum* and the species of Fusarium on soybean belong to the section Elegans, as established by Appel and Wollenweber (1) and modified by Wollenweber (25) in a subsequent study. They are themselves very similar in cultural characters, but can be quite sharply separated from the other species included in the tabulation. When the characters of the species of Fusarium on the cowpea and soybean noted in this table are compared with those in the original descriptions of certain other members of the section Elegans—namely, *F. redolens*, *F. orthoceras*, and *F. conglutinans*, there is plainly no chance of their confusion. *F. redolens* (25) produces no blue sclerotia, and its conidial masses are brownish white; *F. orthoceras* (25) possesses neither sclerotia, sporodochia, nor pionnotes; and *F. conglutinans* (25) is distinguished because of the absence of the typical wine-red to purple colors of the section.

MORPHOLOGICAL AND CULTURAL COMPARISON OF THE FUSARIUM SP. ON SOYBEAN WITH F. TRACHEIPHILUM

Since the studies summarized in Table I do not succeed in distinguishing the species of Fusarium on soybean and cowpea, a more extensive cultural study of these two fungi was made. For this purpose three series of cultures were grown, and the results have been summarized in Table II. Each series contained 10 cultures of each fungus on stem plugs, potato plugs, steamed rice, standard nutrient agar (1.8 per cent agar and 1.0 per cent acid), string-bean hard glucose agar (3 per cent agar, 1.0 per cent acid, and 10 per cent glucose), and oat hard agar (3 per cent agar and 1.0 per cent acid). The cultures were examined when they were 8, 15, 30, 50, and 75 days old.

TABLE II.—*A morphological comparison of the species of Fusarium on soybean and cowpea*

FUSARIUM SP. ON SOYBEAN

Medium.	Macroconidia.	Sporodochia.	Sclerotia.	Color of mycelium.	Character of mycelium.
Standard nutrient agar.	No measurements.	Salmon-colored..	None.....	White.....	Mostly aerial and floccose, becoming appressed in old age.
String-bean agar.....	.....do	Salmon-colored; generally present.	Green.....	.....do.....	Do.
Oat hard glucose agar.	26.6 to 38.6 by 3.69 to 4.92 μ 50 days old.	Flesh-colored....	Dark green..	Mostly lilac; some dark purple.	Cottony.
Steamed rice.....	.....	.....	.....	Reds, pinks, lilacs, purples.	
Potato plugs.....	Normal spores absent.	Salmon-colored; generally present on sclerotia.	Dark green..	Green near sclerotia.	Floccose.
Stem plugs.....	22.5 to 43.6 by 2.87 to 4.11 μ 14 days old.	Salmon-colored; small.	Green; very small; numerous.	White; sometimes green near sclerotia.	Floccose; scant.

F. TRACHEIPHILUM

Standard nutrient agar.	.....	None.....	Flesh-colored..	White.....	Mostly submerged or appressed.
String-bean agar.....	No measurements.	Salmon-colored; few.	Mostly flesh-colored; some green.	.....do.....	Do.
Oat hard glucose agar.	22.5 to 36.9 by 3.8 to 4.42 μ 50 days old.	Flesh-colored..	Dark green and flesh-colored.	Mostly dark purple; some lilac.	Cottony to matted and appressed.
Steamed rice.....	.....	.....	.....	Pinks, reds, lilacs, purples.	
Potato plugs.....	24.6 to 36.9 by 3.28 to 4.42 μ 19 days old.	Salmon-colored; often on sclerotia.	Flesh-colored; often none.	Pinks, lilacs, greens.	Mostly appressed.
Stem plugs.....	.....	Salmon-colored; small; sometimes none.	Green; very small; numerous.	White; sometimes green near sclerotia.	Appressed good growth

No mention is made in Table II of pionnotes or odors, as none were produced in any of the cultures. The microconidia of both strains show a wide variation both in size and shape, but these differences can properly be included in the range of variation. The normal macroconidia of the soybean (fig. 1, *A–G*) and cowpea strains are indistinguishable. The chlamydospores of either strain are terminal or intercalary in or on vegetative filaments and average 6 to 10.25 μ in diameter. The conidiophores are verticillately branched when normal. Sporodochia, although sometimes flesh-colored, are normally salmon-colored. They are not always present on all media, but are formed by each strain either on sclerotia or on mycelia as stromatal bases. Green sclerotia are normally present in both strains. There appear to be some differences in colors produced in substrata, although not very consistent ones, a difference in the character of mycelium until advanced ages of the cultures and gen-

erally, but not always, an absence of flesh-colored sclerotia in the soybean fungus. These differences, however, are not believed to be of sufficient importance to warrant regarding the soybean strain as a distinct species or variety.

In addition to the media employed in Table II, potato hard agar, cornmeal plugs, and string-bean pods were used; but they showed no additional characters of value.

Perithecia have never been observed on the diseased stems; neither have they been obtained in cultures from the surface spores nor from the diseased internal tissues. In fact, the cultural differences between the *Fusarium* sp. on soybean and *Neocosmospora* spp. are as striking as between *Neocosmospora* spp. and the several species of Fusarium causing wilt studied by Higgins (12) and Butler (3).

INOCULATION EXPERIMENTS

From the foregoing morphological and cultural studies, it is evident that the species of Fusarium on soybean is not distinguished from *F. tracheiphilum* by any well-defined differences. Since the possibility existed that they might be separated by biological differences, reciprocal inoculation studies were undertaken to secure additional evidence of their identity.[1]

Plants were therefore grown in pots and flats in the greenhouse and in plots in the field for use in inoculations. The soil used in the pots and flats was a fine, compact, sandy loam, except in the case of one experiment, and was taken from a field in which diseases of cowpeas and soybeans caused by *Fusarium* spp. had never been observed. In certain of these tests, as an added precaution, the soil was partially sterilized by the use of a 2 per cent solution of formaldehyde. The seed were also sterilized in certain experiments by immersion for 15 minutes in commercial sulphuric acid. Since uninoculated plants remained free from disease when these precautions were not employed, their use was discontinued in subsequent tests.

The pots and flats were of sufficient size to permit the plants to grow to maturity.

In determining the percentage of diseased plants, count was made only of those in which it was possible to find discoloration and invasion of the xylem tissues. In case of doubt in this microscopic examination, planted plates were made from the tissues and the subsequent growths studied.

The varieties of soybeans and cowpeas planted for the cross-inoculation experiments were known to be subject in the field to the species of Fusarium on soybean and cowpea, respectively.

EXPERIMENT I.—Twenty-five North Carolina Black cowpea and 25 Mammoth Yellow soybean seedlings, growing in each of two flats

[1] Wollenweber (25, p. 37) says that a consideration of the biological characters is of secondary importance in the determination of species.

in the greenhouse, were each inoculated when from 3 to 6 inches high with spores from sporodochia and with mycelium by introducing the material into incisions in the stems an inch or two below the surface of the soil. All of the plants in one flat were inoculated with the soybean strain of Fusarium and all of those in the other with the cowpea strain. Checks and all inoculated plants except two cowpeas inoculated with the soybean strain and one with the cowpea strain, remained healthy. The test was repeated, using a freshly isolated strain of both organisms; and, since all but one of the plants remained healthy, this method of inoculation was discarded.

EXPERIMENT II (Series 1).—In this experiment the soil in two flats A and B, in the greenhouse was inoculated with pure cultures of *Fusarium* spp. on cowpea and soybean, respectively. These cultures were then incorporated in the upper 4 inches of soil.

The organisms had been grown on pieces of moistened, sterilized cowpea stems until numerous sporodochia had formed. On April 12, 1916, 20 North Carolina Black cowpeas and 20 Mammoth Yellow soybeans were planted in each flat. A third flat, containing uninoculated soil, was planted as a check.

By June 4 a cowpea plant in flat B was noted to be diseased. Others had been observed to be affected by June 15, when all the plants were removed and examined. The results are presented in Table III.

TABLE III.—*Results of growing soybeans and cowpeas in artificially inoculated soil*

Flat.	Organism.	Host.	Total number of plants.	Diseased plants.	
				Number.	Percentage.
A	*Fusarium* sp. on cowpea	Cowpeas	20	6	30
		Soybeans	20	3	15
B	*Fusarium* sp. on soybean	Cowpeas	20	10	50
		Soybeans	20	7	35
C	None (control)	Cowpeas	20	0	0
		Soybeans	20	0	0

EXPERIMENT II (Series 2.)—Since the percentage of diseased plants in series 1 is relatively small, the test was repeated, using another strain of each organism and Clay cowpeas instead of the North Carolina Black variety. Each plant in this test was injured by incision below the surface of the inoculated soil. The period of growth of these plants extended from July 29 to September 1, at which date the plants were fully matured. The results of this series are recorded in Table IV.

EXPERIMENT II (Series 3).—The test in series 2 was duplicated between September 7 and November 20, with no resultant increase in the percentage of infections.

EXPERIMENT III.—Since it was thought that the strain of Fusarium on soybean had to a degree lost its virulence by growth in culture, soy-

bean stems bearing an abundance of sporodochia were macerated and mixed with the soil in two flats. Seed of the Mammoth Yellow variety were planted on May 25. When the experiment was concluded, August 10, only 8 of the 80 soybean plants in these two flats were found to be infected.

TABLE IV.—*Results of growing soybeans and cowpeas in artificially inoculated soil, the plants having been wounded below the surface of the soil*

Flat.	Organism.	Host.	Total number of plants.	Diseased plants.	
				Number.	Percentage.
D	*Fusarium* sp. on cowpea	Cowpeas	20	3	15
		Soybeans	20	3	15
E	*Fusarium* sp. on soybean	Cowpeas	20	6	30
		Soybeans	20	5	25
F	None (control)	Cowpeas	20	0	0
		Soybeans	20	0	0

EXPERIMENT IV.—This experiment was made with soybeans between September 26 and December 1 in an attempt to determine whether the presence of nematodes increases the number of infections. The nematodes were introduced into the soil of large buried pots in root galls from living soybeans free from infection by *Fusarium* sp. The results are presented in Table V.

TABLE V.—*Influence of nematodes on the percentage of infection of soybeans with species of Fusarium*

Organism.	Total number of plants.	Number with nematode galls.	Number with *Fusarium* sp.
Fusarium sp. on cowpea and nematodes	10	10	2
Fusarium sp. on cowpea without nematodes	10	0	0
Fusarium sp. on soybean and nematodes	10	10	3
Fusarium sp. on soybean without nematodes	10	0	2
Nematodes only	10	10	0
None (control)	20	0	0

Only one test of this kind was made; and it is significant to note that there was no increase in infection by the fungus, although the plants in all of the pots into which the galls had been introduced were attacked by eelworms. This experiment was concluded before the plants had matured.

EXPERIMENT V.—Since the more porous types of sandy soil have generally been observed to favor the development of *Fusarium* spp., the cause of wilt diseases, an experiment was performed which was in duplication of Experiment II, series 1, except that the soil consisted of a mixture of three parts of medium-coarse sand and one part of fine sandy

loam and stable manure. The results obtained between September 7 and the close of the experiment, November 20, show considerable increase in the percentage of infection over those in the more compact, fine sandy loam of the preceding experiments even though the cultures used were transfers from cultures nearly 3 months old. Cultures of the strain of *Fusarium* on cowpea were added to flat G, of the species of *Fusarium* on soybean to flat H, and flat J was held as a control. Table VI contains the data on this experiment.

TABLE VI.—*Influence of soil on percentage of infection by Fusarium spp.*

Flat.	Organism.	Host.	Total number of plants.	Diseased plants.	
				Number.	Percentage.
G	*Fusarium* sp. on cowpea	Cowpeas	20	16	80
		Soybeans	20	12	60
H	*Fusarium* sp. on soybean	Cowpeas	20	13	65
		Soybeans	20	12	60
J	None (control)	Cowpeas	20	0	0
		Soybeans	20	0	0

EXPERIMENT VI.—This experiment was designed to confirm the results of inoculations in the greenhouse by inoculations under partially controlled field conditions. Four small plots (No. 26, 27, 28, and 29) on wilt-free soil of the station farm were inoculated as in the previous experiments with pure cultures of the species of Fusarium on soybean; plots No. 59 and 60 were inoculated with the *Fusarium* sp. on cowpea; and two others (100 and 101) were left untreated as controls. Thirty cowpeas and thirty soybeans were planted in each plot on June 10, and the final results noted in Table VII were obtained on September 4.

TABLE VII.—*Results of cross-inoculations in the field*

Plot No.	Organism.	Host.	Total number of plants.	Diseased plants.	
				Number.	Percentage.
26	*Fusarium* sp. on soybean.	Clay cowpeas	30	17	56.6
		Haberlandt soybeans	30	4	13.3
27	do	Clay cowpeas	30	10	33.3
		Tokio soybeans	30	0	0.0
28	do	Clay cowpeas	30	10	33.3
		Mammoth Yellow soybeans	30	8	26.6
29	do	Clay cowpeas	30	15	50.0
		Tar Heel Black soybeans	30	3	10.0
59	*Fusarium* sp. on cowpea.	Clay cowpeas	30	26	86.6
		Tokio soybeans	30	6	20.0
60	do	Clay cowpeas	30	17	56.6
		Mammoth Yellow soybeans	30	6	20.0
100	None (control)	Clay cowpeas	30	0	0.0
		Mammoth Yellow soybeans	30	0	0.0
101	do	Clay cowpeas	30	0	0.0
		Mammoth Yellow soybeans	30	0	0.0

EXPERIMENT VII.—On May 25, 1916, two 100-foot rows of each of the soybean varieties Tokio, Haberlandt, Mammoth Yellow, Medium Yellow, and Virginia were planted in a field which produced a large percentage of wilt in cowpeas in 1914. Two rows of cowpeas were planted in the same plot. By September 1, when all the plants had fully matured, a small percentage of wilted cowpeas had been noted; but no blighted soybeans were found.

Similar data were obtained from observations on cowpeas and soybeans grown in the experimental plot devoted to plant breeding. In this 4-acre plot, three or four rows of soybeans were alternated with three or four rows of cowpeas throughout the field. Some wilt occurred in practically every row of cowpeas in the plot, but careful examinations during the season failed to reveal a single soybean blighted with *Fusarium* sp. among 17 standard varieties and 50 other unnamed selections.

EXPERIMENT VIII.—The field at Red Springs, N. C., in which at least 60 per cent of the Mammoth Yellow soybeans were blighted in 1915, was again planted with this variety on May 23, 1916. In a portion of the field which had been reserved for the purpose, one 54-meter row each of Haberlandt, Mammoth Yellow, Pekin, Black Eyebrow, Medium Yellow, and Tar Heel Black soybeans and a row of Clay cowpeas were planted on June 8. On August 10 the main field and all of the varieties in the test, including the cowpeas, showed considerable blight or wilt, except the Black Eyebrow and the Virginia varieties of soybeans. On August 26 the latter of these varieties was apparently free from disease, but the plants had declined with age to such an extent that the exact determination was doubtful. The Black Eyebrow variety, however, remained free from disease throughout the season.

SUMMARY

(1) A disease of soybeans not previously reported has been studied during the past two years.

(2) The disease is characterized by a chlorosis and shedding of the leaves or leaflets, followed by the death of plants, and is herein called "blight or wilt."

(3) Soybean-blight has been observed in several localities within North Carolina on soils infected with cowpea-wilt.

(4) A species of Fusarium belonging to the section Elegans is the causal organism.

(5) Cultural and morphological studies which are regarded as of primary importance in distinguishing species of Fusarium show that the strain of Fusarium on soybean is identical with the organism producing the wilt of cowpeas.

(6) Reciprocal inoculation experiments with the strains from soybeans and cowpeas show that cross-inoculations can be made. These experiments were conducted in the greenhouse and under field condi-

tions. Pure cultures of the two strains were used in certain of the experiments and inoculum from the natural host in others.

(7) Infection probably occurs through the roots, but nematodes appear not to increase the percentage of blight materially.

(8) The character of the soil appears to influence the percentage of infection, since the largest proportion of diseased plants appeared in coarse sandy soil.

(9) Blight or wilt of soybeans is therefore due to *Fusarium tracheiphilum* Smith.

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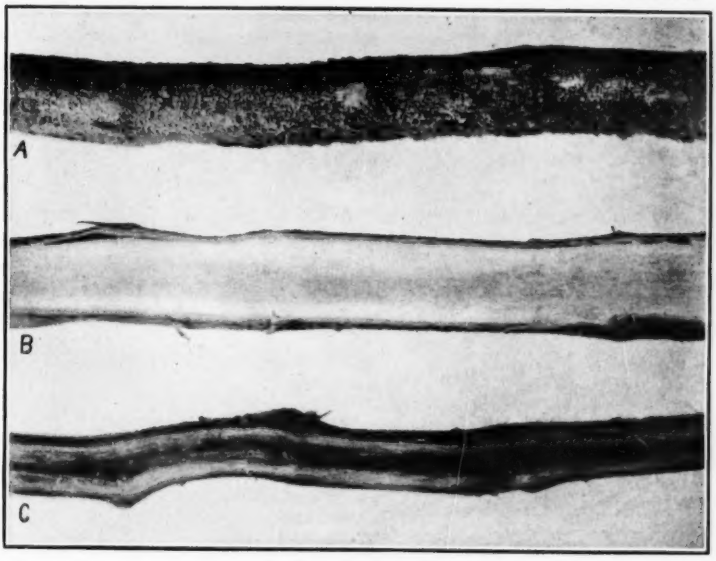
PLATE 95

A.—A diseased stem of soybean, showing the roughened appearance caused by the irregular covering of sporodochia.

B.—Interior of healthy (unstained) stem of soybean.

C.—Interior of diseased (discolored) stem of soybean.

D.—Soybean plants grown out of doors in the same type of clay soil: *D*, healthy; *E*, diseased through the naturally infected soil.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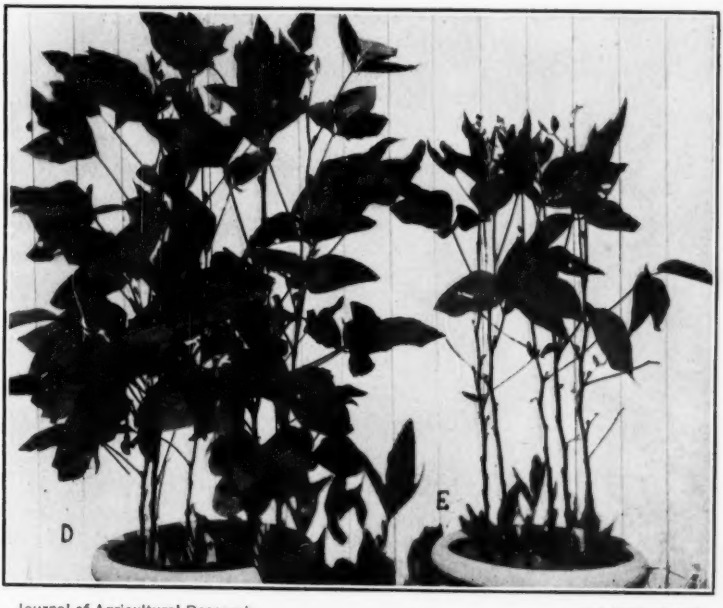
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